The Egyptian Journal of Medical Human Genetics (2015) 16, 11–17

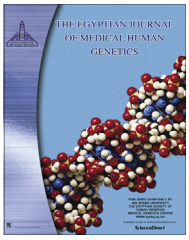
HOSTED BY

ELSEVIER

Ain Shams University

The Egyptian Journal of Medical Human Genetics

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

Association of β-fibrinogen promoter gene polymorphism (−148C/T), hyperfibrinogenemia and ischemic stroke in young adult patients

Imran Imran [a,*], Rusdi Lamsudin [b], Ponpon Idjradinata [c], Tri Hanggono Achmad [d], Amelani Maskoen [d], Samekto Wibowo [b], Harapan Harapan [e]

[a] *Department of Neurology, School of Medicine, Syiah Kuala University, Banda Aceh 23111, Indonesia*
[b] *Department of Neurology, Medical Faculty, Gadjah Mada University, Jogjakarta 55281, Indonesia*
[c] *Department of Paediatrics, Medical Faculty, Padjadjaran University, Bandung 40161, Indonesia*
[d] *Department of Biochemistry, Medical Faculty, Padjadjaran University, Bandung 40161, Indonesia*
[e] *Medical Research Unit, School of Medicine, Syiah Kuala University, Banda Aceh 23111, Indonesia*

Received 11 September 2014; accepted 25 September 2014
Available online 7 November 2014

KEYWORDS

Ischemic stroke;
Fibrinogen;
Hyperfibrinogenemia;
−148C/T FGB polymorphism;
rs1800787.

Abstract *Background:* Single nucleotide polymorphism (SNP) −148C/T which is located in β-fibrinogen gene (FGB) promoter has correlation with fibrinogen levels; however, the association of SNP −148C/T and ischemic stroke in young adult patients is contradictory.

Aim: To determine the association of SNP −148C/T in FGB promoter with plasma fibrinogen levels and ischemic stroke in young adults.

Subjects and methods: In this case-control study, SNP −148C/T among 107 ischemic stroke patients and 94 controls were evaluated by PCR-RFLP with restriction enzyme *Hind*III and confirmed by DNA sequencing. Physical and neurological examinations, brain computed tomography, plasma fibrinogen levels and blood biochemistry tests were assessed within seven days after the onset of symptoms. Genotype distributions and allele frequencies were analyzed by chi-squared test.

Results: This study found that the level of fibrinogen was significantly higher in ischemic stroke group than control (419.2 mg/dL vs. 351.1 mg/dL, $p \leqslant 0.000$) and the level of fibrinogen associated with ischemic stroke (OR, 2.28; 95%CI, 1.28–4.07, $p = 0.005$). Mutant genotypes (CT and TT) and T allele had a significant association with hyperfibrinogenemia (OR, 2.58; 95%CI, 1.39–4.76 and OR, 1.6; 95%CI, 1.60–2.41, respectively) and ischemic stroke (OR, 2.46; 95%CI, 1.37–4.41 and OR, 1.80; 95%CI 1.19–2.73, respectively). In addition, analysis adjusted for other risk factors found that mutant genotypes correlated with hyperfibrinogenemia and ischemic stroke (OR, 2.27; 95%CI, 1.21–4.25 and OR, 2.16; 95%CI, 1.19–3.94, respectively).

* Corresponding author at: Jl. T. Tanoeh Abe, Darussalam, Banda Aceh 23111, Indonesia. Tel.: +62 (0) 85222012940; fax: +62 (0) 651 26090.
E-mail address: imrsps@yahoo.co.id (I. Imran).

Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2014.09.004

Conclusion: There was a significant association between SNP −148C/T and fibrinogen levels, SNP −148C/T and ischemic stroke, and fibrinogen levels and ischemic stroke.

1. Introduction

Elevated plasma fibrinogen, known as hyperfibrinogenemia, is a risk factor for cardiovascular diseases and stroke [1–4] and assessment of the fibrinogen level could help to prevent cardiovascular event [5]. In addition, elevated fibrinogen predicts future ischemic stroke [3] and long-term oral fibrinogen-depleting therapy is beneficial for secondary ischemic stroke prevention [6]. Elevated plasma fibrinogen causes impaired blood coagulation state, increases blood viscosity, slows blood flow, and facilitates platelet aggregation and adhesion to endothelial cells; therefore, it influences blood rheology and plays an important role in atherosclerotic lesion formation that induces cerebrovascular diseases [7].

Fibrinogen, which is a dimeric glycoprotein, consists of three pairs of polypeptide chains: two Aα, Bβ and γ. These six polypeptide chains construct a hexamer (AαBβγ)2 that is joined together with 29 disulfide bonds. Aα, Bβ and γ chains are encoded by 3 different genes, alpha fibrinogen gene (FGA), beta fibrinogen gene (FGB) and gamma fibrinogen gene (FGG), respectively. FGB, which is 8.2 kb long, contains 8 exons [8], and Bβ synthesis is the limiting step in the production of mature fibrinogen [9]. As a consequence, single nucleotide polymorphisms (SNPs) affecting Bβ production would influence plasma fibrinogen levels.

Several SNPs in FGB that determine fibrinogen levels have been identified and one of the most extensively studied is SNP −148C/T (rs1800787) which is located in FGB promoter. Studies have confirmed that SNP −148C/T had association with fibrinogen concentration [10,11]. However, the association of SNP −148C/T and ischemic stroke in adult patients is contradictory [10,12–14]. Therefore, the aim of this study was to determine the association of SNP −148C/T in FGB promoter, fibrinogen levels and ischemic stroke among Indonesian young adult patients.

2. Subjects and methods

2.1. Study setting

This study was a case-control study and the study participants were acute ischemic stroke patients who came for treatment at Dr. Hasan Sadikin Hospital, Bandung. Ischemic stroke diagnostic criteria based on American Stroke Association were applied in this study [15]. The controls were all non-stroke patients – low back pain, radiculopathy, epileptic and myopathy cases – who were age-, sex-, and race-matched, without history of stroke, and not related to the patients. Patients aged over 50 years, had a history of stroke, had sign and/or symptom of infection, had autoimmune disease, obesity, and use of oral anticoagulants were excluded. In both patient and control groups, demographic data and detailed information about cardiovascular risk factors were collected. In all patients, brain computed tomography scan was conducted within seven days of ischemic stroke symptoms onset.

At the admission time, clinical signs and symptoms and neurologic status were assessed and venous blood samples were collected under strictly standardized conditions. Fibrinogen, cholesterol, high-density and low-density lipoprotein, triglyceride, fasting blood sugar, two-hour postprandial blood sugar, and uric acid levels were determined in the blood samples. Fibrinogen was measured as von Clauss principle [16] with Fibri-Prest Automate (Diagnostica Stago, Inc.). For SNP −148C/T genotyping, all blood samples were examined using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and confirmed by DNA sequencing.

This study was conducted with the approval of the Ethics Committee of Medical Faculty, Padjadjaran University, Bandung, Indonesia, No. 09/FKUP-RSHS/KEPK/Kep/EC/2006. The subject recruitment and sample collection were done only after obtaining written informed consent of the participants. The work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans.

2.2. DNA extraction and SNP −148C/T genotyping

DNA isolation and PCR-RFLP were carried out at Faculty of Medicine, Padjadjaran University, Bandung, Indonesia and DNA sequencing confirmation was conducted in 1st BASE Pte. Ltd. Laboratory, Singapore. Briefly, 5 ml peripheral venous blood samples were obtained from patients and controls. DNA was extracted from whole blood using the salting-out method as described previously [17]. Genotyping was carried out as described in previous report [12]. Forward primer was 5′-CCTAACTTCCCATCATTTTGTCCAATTAA-3′, and reverse was: 5′-TGTCGTTGACACCTTGGGA CTTAACTAG-3′. Amplification was performed with 36 cycles (denaturation at 95 °C for 15 s, annealing at 53 °C for 45 s, extension at 72 °C for 30 s) in the 200 μmol/L deoxynucleotide triphosphate, 2.5 mmol/L 1-1 magnesium chloride, and 0.15 units of Taq DNA polymerase. RFLP was performed by digestion of 10 μL of PCR product (362 bp) with 10 μL of *Hind*III restriction enzyme for 24 h. Normally, *Hind*III cut DNA segments into two fragments with a length of 264 bp and 98 bp. Digested products were electrophoresed through ethidium bromide-stained 3% agarose gels. Furthermore, the amplified products, ten samples from each group, were confirmed by DNA sequencing, using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and a 3730 DNA analyzer (Applied Biosystems).

2.3. Statistical analysis

Risk factors of ischemic stroke between case and control groups were analyzed with chi-squared test and Fisher's exact test or Student's *t*-test as appropriate with data. Differences in

genotype, dominant and recessive models, and allele frequencies between ischemic stroke cases and control groups were analyzed with chi-squared test to assess the association of SNP −148C/T with hyperfibrinogenemia and ischemic stroke. Adjusted odd ratios of genotype and allele of SNP −148C/T with hyperfibrinogenemia and ischemic stroke were calculated by logistic regression models. Two-tailed test was used for all significant comparisons to evaluate a statistical *p*-value of $\leqslant 0.05$ as significant.

3. Results

3.1. Sample characteristics and ischemic stroke risk factors

A total of 201 participants, 107 cases (mean age was 42.2 ± 6.53 years old) and 94 controls (mean age was 40.8 ± 9.14 years old) were enrolled in this study. There was no significant difference for sex, age and smoking attitude between case and control groups. Blood biochemistry features and risk factors of ischemic stroke between case and control groups are shown in Table 1. Ischemic stroke patients more often had hypertension ($p \leqslant 0.001$), diabetes ($p \leqslant 0.05$), dyslipidemia ($p \leqslant 0.05$) and hyperuricemia ($p \leqslant 0.05$) compared to controls. As expected, the level of fibrinogen was higher in case than the control group (419.2 ± 118.36 mg/dL vs. 351.1 ± 96.99 mg/dL, $p < 0.001$). In this study, fibrinogen level > 375 mg/dL was used as cut off point of hyperfibrinogenemia. By using this cut off point, 51.8% of cases had hyperfibrinogenemia and 34.4% of controls had hyperfibrinogenemia. Analysis found that there was a strong association between hyperfibrinogenemia status and ischemic stroke (OR, 2.28; 95%CI, 1.28–4.07, $p = 0.005$).

3.2. Association of SNP −148C/T FGB and fibrinogen levels

The SNP −148C/T was genotyped by PCR-RFLP with the genotyping success rates of 95%–100% and repeatability rates of 98%–100%. DNA sequencing was used to further confirm the genotypes for SNP −148C/T from both groups (10% samples each group) with the concordance rates between RFLP and DNA sequencing being 98–100%. The location of SNP −148C/T is presented in Fig. 1A. The amplified PCR products (362 bp) were digested with *Hind*III and electrophoresed through 3% agarose gels. Two fragments of 264 and 98 bp were categorized as homozygous CC genotype (normal genotype), whereas a 362 bp fragment was categorized as homozygous TT genotype (homozygote mutant genotype). Three fragments of 362, 264 and 98 bp were categorized as heterozygous CT (heterozygous mutant genotype) (Fig. 1B).

Among cases and controls, the level of fibrinogen in mutant genotypes (CT and TT) was significantly higher than normal genotype (CC) (404.5 ± 123.90 mg/dL vs. 359.1 ± 88.72 mg/dL, $p = 0.006$) (Table 2). By using 375 mg/dL as cut off point hyperfibrinogenemia, there were 83 hyperfibrinogenemia and 118 normal fibrinogen level among patients and controls. On further analysis, this study found that hyperfibrinogenemia had a significant association

Table 1 Sample characteristics and ischemic stroke risk factors between case ($n = 107$) and control ($n = 94$) groups.

Characteristic	Ischemic stroke *n* (%)	Control *n* (%)	OR (95%CI)	*p*
Sex, men[a]	49 (45.8)	37 (39.4)	1.3 (0.72–2.31)	0.219
Average age[b]	42.2 (±6.53)	36.8 (±9.14)		0.076
Smoking[a]	46 (43.0)	31 (33.0)	1.5 (0.91–2.73)	0.095
Diabetes mellitus[a]	14 (13.1)	5 (5.3)	2.6 (0.91–7.70)	0.049*
Fasting blood sugar (mean, mg/dL)[b]	109.3 (±49.62)	95.6 (±33.13)		0.025*
Two-hour PP sugar (mean, mg/dL)[b]	134.5 (±65.44)	117.8 (±46.26)		0.041*
Hypertension[a]	75 (70.1)	14 (14.9)	13.4 (6.61–27.4)	0.000**
Systolic blood pressure (mean, mm Hg)[b]	153.2 (±33.18)	122.4 (±13.91)		0.000**
Diastolic blood pressure (mean, mm Hg)[b]	92.6 (±15.98)	79.7 (±7.97)		0.000**
Hypercholesterolemia[a]	43 (40.2)	23 (24.5)	2.1 (1.13–3.82)	0.013*
Total cholesterol (mean, mg/dL)[b]	194.5 (±43.29)	192.7 (±39.99)		0.765
High low-density lipoprotein[a]	20 (18.7)	8 (8.5)	2.4 (1.04–5.92)	0.029*
Low-density lipoprotein (mean, mg/dL)[b]	119.2 (±39.68)	98.6 (±46.28)		0.001*
Low high-density lipoprotein[a]	77 (72.0)	34 (36.2)	4.5 (2.50–8.23)	0.000**
High-density lipoprotein (mean, mg/dL)[b]	43.9 (±16.33)	59.2 (±14.19)		0.000**
Hypertriglyceridemia[a]	35 (32.7)	9 (9.6)	4.6 (2.11–10.21)	0.000**
Triglyceride (mean, mg/dL)[b]	139.1 (±56.39)	115.6 (±72.21)		0.010*
Hyperuricemia[a]	11 (10.3)	1 (1.1)	10.7 (1.33–84.26)	0.005*
Blood uric acid (mean, mg/dL)[b]	5.8 (±2.50)	4.8 (±0.87)		0.000**
Hyperfibrinogenemia[a]	54 (51.8)	29 (34.3)	2.28 (1.28–4.07)	0.005*
Serum fibrinogen (mean, mg/dL)[b]	419.2 (±118.36)	351.1 (±96.99)		0.000**

PP: postprandial.
[a] Chi-squared test analysis.
[b] Student's *t*-test analysis.
* Statistically significant at $\leqslant 0.05$.
** Statistically significant at $\leqslant 0.001$.

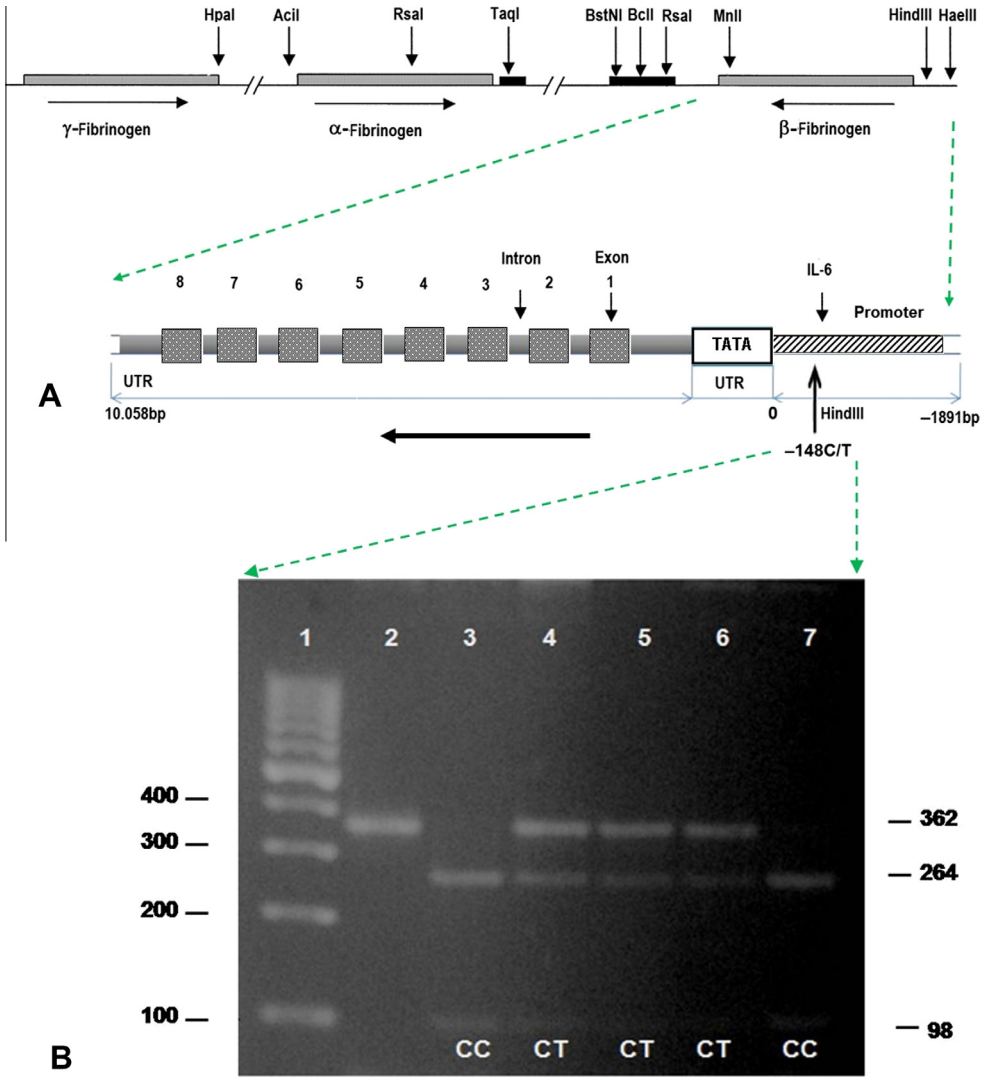


Figure 1 (A) Localization of SNP −148C/T FGB in a schematic map of the fibrinogen gene cluster. (B) The PCR-RFLP product electrophoresis of SNP −148C/T FGB. Description: Lane 1: DNA marker ladder 100, Lane 2: uncut PCR-RFLP product of 362 bp, Lanes 3 and 7: CC genotype, and Lanes 4–6: CT genotype. Homozygous TT genotype characterized with a 362 bp fragment (not present in (B)).

with mutant genotype (OR, 2.58; 95%CI, 1.39–4.76) and T allele (OR, 1.6; 95%CI, 1.60–2.41) (Table 3). Furthermore, logistic regression analysis (adjusted for sex, age, smoking status, diabetes mellitus, hypertension, dyslipidemia and blood uric acid level) found that hyperfibrinogenemia was correlated significantly with mutant genotype (OR, 2.27; 95%CI, 1.21–4.24) (Table 3).

3.3. Association of SNP −148C/T FGB and ischemic stroke

There were significant differences in the genotype distributions and allele frequencies between case and control groups (Table 4). In addition, there was a strong association between mutant genotypes (CT and TT) and ischemic stroke (OR, 2.46; 95%CI, 1.37–4.41) and between mutant allele (T allele) and ischemic stroke (OR, 1.80; 95%CI, 1.19–2.73). Furthermore, logistic regression analysis found that mutant genotype and mutant allele were significantly correlated with ischemic stroke (OR, 2.16; 95%CI, 1.19–3.94, and OR, 1.68; 95%CI, 1.10–2.57 respectively) (Table 4).

4. Discussion

Our previous studies have revealed that SNPs in specific genes influence the human susceptibility either to infectious diseases [18–21] or other condition related infections [22]. In this study we tried to determine the association between SNP −148C/T and hyperfibrinogenemia and ischemic stroke. The genetic influence on FGB has been extensively studied, because Bβ synthesis is the limiting step in the production of mature fibrinogen [9]. One of the SNPs present in FGB, −148C/T, is located in FGB promoter (see Fig. 1A). This study found that the levels of fibrinogen from persons who had mutant T allele (CT and TT) in −148C/T were significantly higher than persons who had normal allele (C allele). Therefore, this study con-

Table 2 The association of SNP −148C/T and fibrinogen levels among ischemic stroke and control groups (n = 201).

Genotype and allele	Mean of fibrinogen level (mg/dL)	p
Genotype		0.014*
CC	359.1 (±139.22)	
CT	408.5 (±121.24)	
TT	382.1 (±88.72)	
Dominant model		0.006*
CC	359.1 (±88.72)	
CT + TT	404.5 (±123.90)	
Recessive model		
CC + CT	387.9 (±111.29)	0.833
TT	382.1 (±139.22)	
Allele		
C	379.4 (±111.00)	0.061
T	401.6 (±123.20)	

* Statistically significant at ⩽0.05.

firmed that T allele in −148C/T FGB is related to higher fibrinogen levels (hyperfibrinogenemia) as mentioned previously [10,11,23,24]. Meta-analysis study also found that fibrinogen levels of allele T carrier (TT and CT) in −148C/T were higher than C allele among ischemic stroke patients in Han Chinese population [25]. In addition, in the general population, the level of plasma fibrinogen is significantly higher in the groups of genotypes −148C/T and TT than CC [13].

One of the possible reasons is because SNP −148C/T is close to the responsive elements of interleukin-6 (IL-6), the regulating element of the activating repression of fibrinogen gene transcription [7] (Fig. 1A). Sequences responsive element of IL-6 including CCAAT box/enhancer-binding protein (C/EBP)-binding site, hepatocyte nuclear factor (HNF) and IL6-responsive element (IL-6 RE), are present in FGB promoter region [26]. Verschuur et al. [27] found that SNP −148C/T, which is present between HNF-3-binding site and C/EBP-binding site, influences fibrinogen promoter activity in response to IL6.

van Goor et al. [14] argue that a high level of fibrinogen in the acute phase of ischemic stroke probably represents an acute phase response. They found that there were significant differences between fibrinogen levels in patients and controls during acute onset of ischemic stroke, but no difference after three months of acute onset. Although, fibrinogen levels might reflect inflammatory process that is caused by ischemic stroke, fibrinogen levels might contribute directly to ischemic stroke development because it contributes to generation and progression of atherosclerosis -inducing platelet aggregation, decreasing blood viscosity and increasing endothelial cell injury- and affects the stability and structure of the plaque [7,27].

The important role of SNP −148C/T also has been studied related to other cardiovascular diseases. Previous study found that the presence of the T allele has been associated with angiographic severity of atherosclerosis in postmyocardial infarction [28] and the TT genotype has been associated with carotid atherosclerosis [29]. Furthermore, recent study from a meta-analysis including 15,055 subjects in Chinese population found that there was a significant association between SNP −148C/T and coronary artery disease under allelic, recessive, dominant and homozygous genetic models [30]. This study found that mutant genotypes (CT and TT) and mutation allele (T) in SNP −148C/T had a significant association with ischemic stroke. This evidence suggests that the T allele is a risk factor for ischemic stroke at young age. A meta-analysis conducted in China, showed that SNP −148C/T contributed to the vulnerability of ischemic stroke in Han Chinese and allele T carrier increased the risk of ischemic stroke about 32% compared with the wild CC homozygote [25].

Table 3 The association of SNP −148C/T and hyperfibrinogenemia.

Genotype and allele	Hyperfibrinogenemia		OR (95%CI)	p
	Yes (n%)	No (n%)		
Genotype			-	0.012*
CC	22 (26.5)	55 (46.6)		
CT	53 (63.9)	52 (44.1)		
TT	8 (9.6)	11 (9.3)		
Dominant model			2.58 (1.39–4.76)	0.002*
CC	22 (25.0)	55 (46.6)		
CT + TT	61 (75.0)	63 (53.4)		
Recessive model			1.04 (0.39–2.70)	0.562
CC + CT	75 (90.4)	107 (90.7)		
TT	8 (9.6)	11 (9.3)		
Allele			1.60 (1.06–2.41)	0.017*
C	96 (57.8)	162 (68.6)		
T	70 (42.2)	74 (31.4)		
Logistic regression model[a]				
C vs. T			1.45 (0.95–2.21)	0.086
CC vs. CT + TT			2.27 (1.21–4.25)	0.011*

** Statistically significant at ⩽0.001.
[a] Adjusted for sex, age, smoking status, diabetes mellitus, hypertension, dyslipidemia and blood uric acid level.
* Statistically significant at ⩽0.05.

Table 4 The association of SNP −148C/T and ischemic stroke.

Genotype and allele	Ischemic stroke		OR (95%CI)	*p*
	Yes (*n*%)	No (*n*%)		
Genotype			-	0.012*
CC	31 (29.0)	46 (48.9)		
CT	63 (58.9)	42 (44.7)		
TT	13 (12.1)	6 (6.4)		
Dominant model			2.46 (1.37–4.41)	0.002*
CC	31 (28.0)	46 (49.0)		
CT + TT	76 (72.0)	48 (51.0)		
Recessive model			2.03 (0.74–5.57)	0.124
CC + CT	94 (87.9)	88 (93.6)		
TT	13 (12.1)	6 (6.4)		
Allele			1.80 (1.19–2.73)	0.004*
C	124 (57.9)	134 (71.3)		
T	90 (42.1)	54 (28.7)		
Logistic regression model[a]				
C vs. T			1.68 (1.10–2.57)	0.017*
CC vs. CT + TT			2.16 (1.19–3.94)	0.012*

[a] Adjusted for sex, age, smoking status, diabetes mellitus, hypertension, dyslipidemia and blood uric acid level.
* Statistically significant at $\leqslant 0.05$.

A study found that there was no relationship between SNP −148C/T and ischemic stroke in their total sample, but in the subgroup of smoking population the odd ratio of allele T carriers to CC homozygotes reached 4.85 [30]. In addition, persons with mutant genotype in SNP −148C/T (combined with other gene polymorphisms) had an elevated incidence of ischemic stroke and the incidence was increased with combination with other risk factors such as smoking and alcohol consumption [31]. It seems that interaction of SNP −148C/T and environmental factors is important. However, other studies found that SNP −148C/T had no association with ischemic stroke [14] and other cardiovascular events [12]. These differences might due to racial differences as described previously [32].

Furthermore, this study found that fibrinogen level was associated with ischemic stroke in young age. Inflammatory factors such as fibrinogen are consistently associated with an increased risk of cardiovascular diseases including stroke. Previous data revealed that fibrinogen level was associated with stroke [2,33–35] and increased fibrinogen levels were associated with poor outcome [33,36] and mortality of ischemic stroke [34]. Levels of fibrinogen were strongly associated with ischemic stroke severity in almost all studied populations [36]. In addition, it is clear that modification of prothrombotic factors in ischemic stroke is beneficial [37]. Therefore, if the elevation of fibrinogen is controlled by genetic factor; preventive efforts to reduce or normalize plasma fibrinogen levels might be important to minimize ischemic stroke incidence in young age adults.

There are several limitations of this study. This study did not obtain prospective data examining the effect of SNP −148C/T on long-term clinical outcome. In addition, the controls were patients who visited hospital with non-ischemic stroke; therefore this approach might cause hospital control bias. Finally, this study did not measure the level of fibrinogen in convalescent phases; therefore, this study was unable to answer debatable question whether fibrinogen is a risk factor of ischemic stroke or merely a marker of acute phase reactions.

5. Conclusion

This study found that mutant genotypes (TT and CT) in −148C/T FGB had a significant correlation with hyperfibrinogenemia and ischemic stroke compared with normal genotype (CC), and a high level of fibrinogen was associated with ischemic stroke in young adult patients. It seems that hyperfibrinogenemia is a predictor factor for ischemic stroke in young adults.

Conflict of interest

We have no conflict of interest to declare.

Acknowledgments

The authors extend thanks to Eijkman Institute for Molecular Biology Jakarta. This study was supported by Riset Pembinaan Iptek Kedokteran (Risbiniptekdok) grant from The Indonesian Directorate General of Higher Education (DIKTI) and Indonesia Managing Higher Education for Relevance and Efficiency (IMHERE) grant.

References

[1] Chuang SY, Bai CH, Chen WH, Lien LM, Pan WH. Fibrinogen independently predicts the development of ischemic stroke in a Taiwanese population: CVDFACTS study. Stroke 2009;40(5):1578–84.

[2] Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant meta-analysis. JAMA 2005;294:1799–809.

[3] Kofoed SC, Wittrup HH, Sillesen H, Nordestgaard BG. Fibrinogen predicts ischaemic stroke and advanced atherosclerosis but not echolucent, rupture-prone carotid plaques: the Copenhagen City Heart Study. Eur Heart J 2003;24(6):567–76.
[4] Rothwell P, Howard SC, Power DA, Gutnikov SA, Algra A, van Gijn J, et al. Fibrinogen concentration and risk of ischemic stroke and acute coronary events in 5113 patients with transient ischemic attack and minor ischemic stroke. Stroke 2004;35:2300–5.
[5] Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P. C-reactive protein, fibrinogen, and cardiovascular disease prediction. N Engl J Med 2012;367(14):1310–20.
[6] Cao YJ, Zhang X, Wang WH, Zhai WQ, Qian JF, Wang JS, et al. Oral fibrinogen-depleting agent lumbrokinase for secondary ischemic stroke prevention: results from a multicenter, randomized, parallel-group and controlled clinical trial. Chin Med J (Engl) 2013;126(21):4060–5.
[7] Kamath S, Lip GY. Fibrinogen: biochemistry, epidemiology and determinants. QJM 2003;96(10):711–29.
[8] Kant JA, Fornace Jr AJ, Saxe D, Simon MI, McBride OW, Crabtree GR. Evolution and organization of the fibrinogen locus on chromosome 4: gene duplication accompanied by transposition and inversion. Proc Natl Acad Sci USA 1985;82:2344–8.
[9] Yu S, Sher B, Kudryk B, Redman CM. Fibrinogen precursors order of assembly of fibrinogen chains. J Biol Chem 1984;259:10574–81.
[10] Jood K, Danielson J, Ladenvall C, Blomstrand C, Jern C. Fibrinogen gene variation and ischemic stroke. J Thromb Haemost 2008;6(6):897–904.
[11] Wypasek E, Stepien E, Kot M, Plicner D, Kapelak B, Sadowski J, et al. Fibrinogen beta-chain -C148T polymorphism is associated with increased fibrinogen, C-reactive protein, and interleukin-6 in patients undergoing coronary artery bypass grafting. Inflammation 2012;35(2):429–35.
[12] Blake G, Schmitz C, Lindpaintner K, Ridker PM. Mutation in the promoter region of the B-fibrinogen gene and the risk of future myocardial infarction, stroke and venous thrombosis. Eur Heart J 2001;22:2262–6.
[13] Liang L, Sun C, Xiao F, Tang XL, Chen XD, Zhou DF, et al. Nine polymorphisms of fibrinogen gene and their association with plasma fibrinogen levels in Hainan Han population. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2005;22(4):457–61 [in Chinese].
[14] van Goor MPJ, Gómez-Garcıa EB, Leebeek FWG, Brouwers GJ, Koudstaal PJ, Dippe DWJ. The -148 C/T fibrinogen gene polymorphism and fibrinogen levels in ischaemic stroke: a case-control study. J Neurol Neurosurg Psychiatry 2005;76(1):121–3.
[15] Jauch EC, Saver JL, Adams Jr HP, Bruno A, Connors JJ, Demaerschalk BM, et al. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2013;44(3):870–947.
[16] Mackie IJ, Kitchen S, Machin SJ. Lowe GD; haemostasis and thrombosis task force of the British committee for standards in haematology. Guidelines on fibrinogen assays. Br J Haematol 2003;121(3):396–404.
[17] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.
[18] Harapan H, Fajar JK, Wahyuniati N, Anand JR, Nambaru L, Jamil KF. Non-HLA gene polymorphisms and their implication on dengue virus infection. Egypt J Med Hum Genet 2013;14:1–14.
[19] Harapan H, Fajar JK, Khalilullah SA, Winardi W, Jamil KF. Genetic polymorphisms of HLA and HLA-related proteins: implications on dengue virus infection. J Mosq Res 2013;3(1):1–10.
[20] Khalilullah SA, Harapan H, Hasan NA, Winardi W, Ichsan I, Mulyadi M. Host genome polymorphisms and tuberculosis infection: what we have to say? Egypt J Chest Dis Tuberc 2014;63:173–85.
[21] Syukri M, Sja'bani M, Soesatyo MHNE, Astuti I, Imran I, Harapan H. The promoter region (G-800A and C-509T) polymorphisms of transforming growth factor-β1 gene among young women with recurrent urinary tract infection. Egypt J Med Hum Genet 2014;15:125–30.
[22] Tumangger H, Jamil KF. Contribution of genes polymorphism to susceptibility and outcome of sepsis. Egypt J Med Hum Genet 2010;11:97–103.
[23] Fu Y, Wei X, Ni PH, Ying YY, Song YY, Chen SD. The relationship between the five beta-fibrinogen gene polymorphisms and cerebral infarction. Zhonghua Nei Ke Za Zhi 2005;44(12):914–7 [in Chinese].
[24] Guo X, Zhang D, Zhang X. Fibrinogen gene polymorphism (Beta-148C/T) in Uygur patients with cerebral infarction. Neurol Res 2009;31(4):381–4.
[25] Chen XC, Xu MT, Zhou W, Han CL, Chen WQ. A meta-analysis of relationship between beta-fibrinogen gene −148C/T polymorphism and susceptibility to cerebral infarction in Han Chinese. Chin Med J (Engl) 2007;120(13):1198–202.
[26] Soria JM, Almasy L, Souto JC, Buil A, Lathrop M, Blangero J, et al. A genome search for genetic determinants that influence plasma fibrinogen levels. Arterioscler Thromb Vasc Biol 2005;25(6):1287–92.
[27] Verschuur M, de Jong M, Felida L, Moniek PM, de Maat MPM, VoS HL. A hepatocyte nuclear factor-3 site in the fibrinogen beta promoter is important for interleukin 6-induced expression, and its activity is influenced by the adjacent −148C/T polymorphism. J Biol Chem 2005;280(17):16763–71.
[28] Behague I, Poirier O, Nicaud V, Evans A, Arveiler D, Luc G, et al. Beta fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. The ECTIM study. Etude Cas-Temoins sur l'Infarctus du Myocarde. Circulation 1996;93:440–9.
[29] Schmidt H, Schmidt R, Niederkorn K, Horner S, Becsagh P, Reinhart B, et al. Beta-fibrinogen gene polymorphism (C148T) is associated with carotid atherosclerosis: results of the. Austrian Stroke Prevention Study. Arterioscler Thromb Vasc Biol 1998;18:487–92.
[30] Li YJ, Huo Y, Gao XG, Teng ZP, Ren ZH, Pan KF. A statistical study on the relationship between gene polymorphisms of β-fibrinogen −148C/T, N5, N10- methylene-tetra-hydrofolic acid reductase 677C/T and cerebral infarction. Chin J Neuroimmunol Neurol 2005;12:363–8 [in Chinese].
[31] Gao X, Yang H, ZhiPing T. Association studies of genetic polymorphism, environmental factors and their interaction in ischemic stroke. Neurosci Lett 2006;398(3):172–7.
[32] Cook D, Cappuccio FP, Atkinson RW. Ethnic differences in fibrinogen levels: the role of environmental factors and the beta-fibrinogen gene. Am J Epidemiol 2001;153:799–806.
[33] del Zoppo G, Levy DE, Wasiewski WW, Pancioli AM, Demchuk AM, Trammel J, et al. Hyperfibrinogenemia and functional outcome from acute ischemic stroke. Stroke 2009;40:1687–91.
[34] Turaj W, Slowik A, Dziedzic T, Pulyk R, Adamski M, Strojny J, et al. Increased plasma fibrinogen predicts one-year mortality in patients with acute ischemic stroke. J Neurol Sci 2006;246(1–2):13–9.
[35] Woodward M, Lowe GD, Campbell DJ, Colman S, Rumley A, Chalmers J, et al. Associations of inflammatory and hemostatic variables with the risk of recurrent stroke. Stroke 2005;36(10):2143–7.
[36] Di Napoli M, Papa F. Should neurologists measure fibrinogen concentrations? J Neurol Sci 2006;246:5–9.
[37] Alvarez-Perez FJ, Castelo-Branco M, Alvarez-Sabin J. Usefulness of measurement of fibrinogen, D-dimer, D-dimer/fibrinogen ratio, C reactive protein and erythrocyte sedimentation rate to assess the pathophysiology and mechanism of ischaemic stroke. J Neurol Neurosurg Psychiatry 2011;82:986–92.